Arabian Journal of Chemistry (2019) 12, 1308–1314

King Saud University

Arabian Journal of Chemistry

www.ksu.edu.sa
www.sciencedirect.com

SPECIAL ISSUE: ENVIRONMENTAL CHEMISTRY

Biochemical response of *Cupressus sempervirens* to cement dust: Yields and chemical composition of its essential oil

Karim Hosni [a,*], Imed Hassen [b], Yacine M'Rabet [a], Hervé Casabianca [c]

[a] *Laboratoire des Substances Naturelles, Institut National de Recherche et d'Analyse Physico-chimique, Biotechpôle de Sidi Thabet, 2020, Tunisia*
[b] *Laboratoire des Méthodes et Techniques Analytiques, Institut National de Recherche et D'Analyse Physico-chimique (INRAP), Biotechpole de Sidi Thabet, 2020, Tunisia*
[c] *Institut des Sciences Analytiques, Département Service Central d'Analyse, Centre National de Recherche Scientifique (CNRS), 5 Rue de la Doua, 69100 Villeurbanne, Lyon, France*

Received 11 July 2013; accepted 29 October 2014
Available online 18 November 2014

KEYWORDS

Cement dust;
Cupressus sempervirens;
Essential oil;
Monoterpenes;
Environmental pollution

Abstract The effects of cement dust on the yield and chemical composition of the essential oil were investigated in *Cupressus sempervirens*. Exposure to cement dust resulted in a significant increase in the essential oil yields. Significant factory distance-related changes in qualitative and quantitative composition of the essential oil were observed. Increasing pollution with dust increased the content of monoterpene hydrocarbons concomitant to increase of α-pinene, suggesting a redirection of the secondary metabolism of *C. sempervirens* towards biosynthesis of monoterpenes. In contrast, oxygenated monoterpenes and sesquiterpene hydrocarbons were strongly reduced. These results provide an overall picture of the different responses of monoterpenes and sesquiterpenes to air pollution caused by cement dust. They also reveal the suitability of using *C. sempervirens* in the creation of green areas around cement factories and encourage the use of dusted plants as potential source of valuable natural products.

1. Introduction

Terpenes are the most functionally and structurally diverse group of plant secondary metabolites. They are thought to play a pivotal role in essential physiological and biological processes such as respiration, photosynthesis and regulation of growth and development (Phillips et al., 2008). They are also involved in plant–environment interactions by playing roles in pollinator attraction, defence and plant–plant communications

* Corresponding author. Tel.: +216 71537666; fax: +216 71537688.
E-mail addresses: hosni_karim@voila.fr, karim.hosni@inrap.rnrt.tn (K. Hosni).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.arabjc.2014.10.042
1878-5352

(Mahmoud and Croteau, 2002). Because of their odoriferous principles and their intriguing biological activities such as antioxidant, antimicrobial, antifungal, antiviral, anti-inflammatory, anti-carcinogenic and antimalarial among others (Bakkali et al., 2008), terpenes find extensive industrial applications as flavouring agents, topical medicine, perfumes and cosmetics (Aharoni et al., 2005). With regard to extensive demand and commercial interests of essential oils, numerous aromatic species have been exhaustively studied and substantial insight into the chemical composition and the metabolism of their essential oils has been published. Member of the Cupressaceae family are among the potential producers of essential oils and some of them namely *Cupressus lusitanica* and *Cupressus sempervirens* have been studied in detail (Cool et al., 1998; De Alwis et al., 2009). The basic constituent of the oil of *C. sempervirens* is α-pinene, to which are attributed the antioxidant (Wang et al., 2008), antimicrobial (Jiang et al., 2011; Ojeda-Sana et al., 2013), antifungal (Chang et al., 2008), anti-inflammatory (Bae et al., 2012), antimalarial, anticoagulative (Yang et al., 2011), phytotoxic (Abrahim et al., 2003), cytotoxic (Matsuo et al., 2011) and myorelaxant (Lima et al., 2010) properties.

As commonly happens for many species, the yield and chemical composition of the essential oil bearing plants have been shown to be strongly influenced by the genetic (Hosni et al., 2010, 2013) and environmental factors such as salinity, moisture, temperature, light intensity, nutrition and photoperiod (Banchio et al., 2005; Harrathi et al., 2012). However, the effect of air pollution in general and cement dust in particular on the essential oil constitution and production has not received much attention. This ‘‘silent threat’’ is generally associated with disruption of physiological and biochemical processes, particularly those including the biosynthesis of essential oils (Kupcinskiene et al., 2008; Dziri and Hosni, 2012). These authors reported that the exposure to cement dust resulted in profound qualitative and quantitative changes in the chemical composition of the essential oils of *Pinus sylvestris* and *Pinus halepensis* needles. From compositional stand point, long term exposure to cement dust was associated with higher concentration of γ-terpinene and caryophyllene oxide in *P. sylvestris* needles (Kupcinskiene et al., 2008), while a significant decrease in all components of the oil of *P. halepensis* was observed in the needles collected from plants grown in heavy polluted sites (Dziri and Hosni, 2012). Based on these antecedents, it seems that the impact of cement dust on essential oil constituents depend mostly on plant species.

Keeping in view these aspects, the present study was conducted to assess the effects of cement dust on yields and chemical composition of the essential oil from *C. sempervirens* foliage. These results should contribute to better understanding of the biochemical response of this species to environmental contamination by cement dust and will help to estimate the suitability of using *C. sempervirens* in creating green areas around cement factories.

2. Materials and methods

2.1. Sampling sites

The study area is located at the close vicinity of the cement factory of Bir Mcherga (north-eastern Tunisia) (Fig. 1). The experimental design was adopted from Bačić et al. (1999) and Yilmaz and Zengin (2004). The geographic positions of the polluted sites (site 1, 2) and the unpolluted site are determined with Magellan Pro handheld global positioning system (GPS) and they are as follows: Site 1 situated at the distance of 50 m from the cement factory (latitude 36°, 31′, 1,97″ (N); longitude 10°, 01′, 18,33″ (E); altitude 157.35 m), and site 2 situated at 800 m from the cement factory (latitude 36°, 30′, 43,140″ (N); longitude 10°, 01′, 8,23″ (E); altitude 142.3 m). Environmental monitoring studies show that the main pollutants in the aforementioned sites are CaO, (SO_x), (NO_x), (CO_x) and fluorides (RNEE 2001). These pollutants are emitted in large part by the cement factory and to a less extent by the road traffic. The pH of the soil ranged from 8.7 to 8.4 in site 1 and site 2, respectively. The unpolluted site is situated at 5 km in the east of the cement factory: Ecole Supérieure d′Agriculture de Mograne (latitude 36°, 25′, 38″ (N); longitude 10°, 05′, 41″ (E); altitude 149 m), at the western foot of the Zaghouan mountain. It's only affected by local emission caused by home furnace and the low intensive road traffic (Dziri and Hosni, 2012). The pH of the soil was 7.6. The climate of Bir Mcherga is mild and continental with hot and dry summer while, it is cold and humid in winter. The most frequent annual wind direction is the east wind.

At each study site, leaves of *C. sempervirens* were collected from 15 trees. To avoid daily variations of the essential oil components, the sampling was done as far as possible in the same time of the day (11 h–13 h). The harvested material was dried at room temperature (20 ± 2 °C) for one week, ground by using a Retsch blender mill (Normandie-Labo, Normandy, France), sifted through 0.5 mm mesh screen to obtain a uniform particle size and subsequently assayed for their essential oil composition.

2.2. Essential oils isolation

The air dried leaves (100 g) were submitted to hydrodistillation for 3 h using a Clevenger-type apparatus. The oils obtained were dried over anhydrous sodium sulphate (Na_2SO_4) and stored in amber and air-tight sealed vials at 0 °C until required.

2.3. Analysis of essential oils

Gas chromatography analyses were carried out on a Shimadzu HRGC-2010 gas chromatograph (Shimadzu Corporation, Kyoto, Japan) equipped with flame ionization detector (FID), Auto-injector AOC-20i and auto-sampler AOC-20s. Separation of volatile components was performed by using an apolar column Rtx-1 (30 m × 0.25 mm, 0.32 μm film thickness). The oven temperature was held at 50 °C for 10 min then programmed at 2 °C/min to 190 °C. The injector and detector temperature were programmed at 230 °C. The flow of the carrier gas (N_2) was 1.6 mL/min and the split ration was 1:20. Injection volume for all samples was 0.5 μL of diluted oils in *n*-pentane (LabScan, Dublin, Ireland).

The GC–MS analyses were performed on a gas chromatograph HP 6890 (II) interfaced with a HP 5973 mass spectrometer (Agilent Technologies, Palo Alto, Ca, USA) with electron impact ionization (70 eV). A HP-5MS capillary column (60 m × 0.25 mm, 0.25 μm film thickness) was used. The column temperature was programmed to rise from 40 to

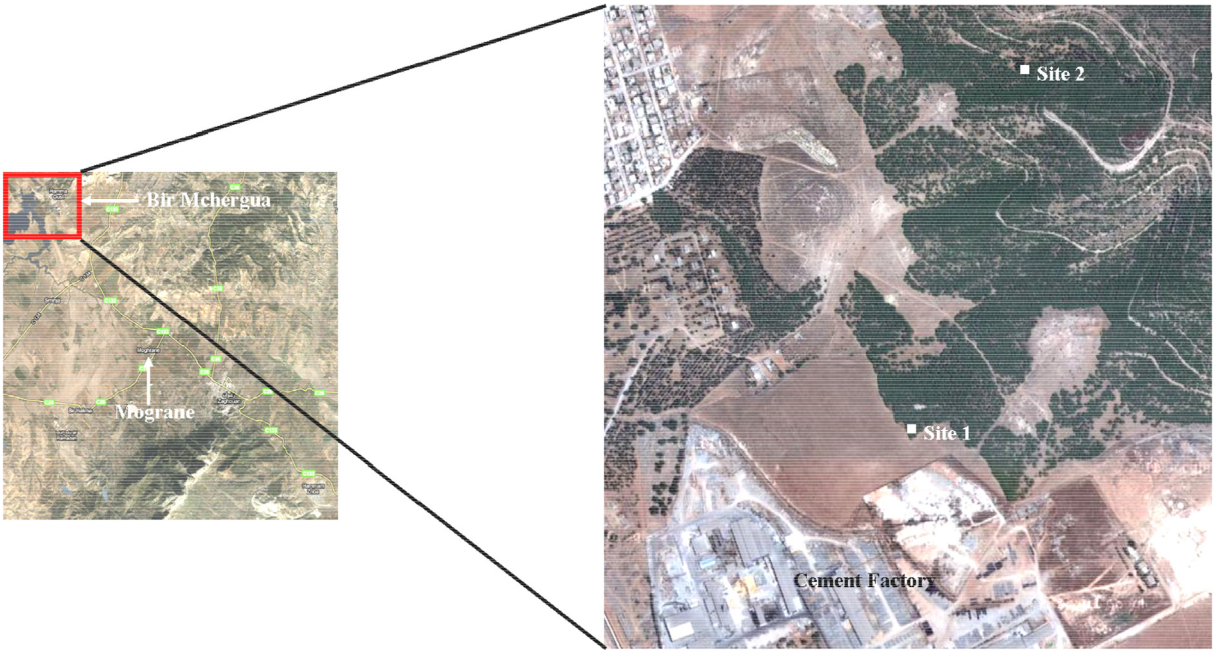

Figure 1 Location of the study area (left) and the sampling sites in the polluted zone (right).

280 °C at a rate of 5 °C/min. The carrier gas was helium with a flow rate of 1.2 mL/min. Scan time and mass range were 1 s and 50–550 m/z, respectively.

The volatile compounds were identified by comparison of their retention indices relative to (C_7–C_{20}) *n*-alkanes with those of literature (Paolini et al., 2005; Hosni et al., 2010) and/or with those of authentic compounds available in our laboratory, and by matching their mass spectral fragmentation patterns with corresponding data (Wiley 275.L library) and other published mass spectra (Adams, 2001) as well as by comparison of their retention indices with data from the Mass Spectral Library "Terpenoids and Related Constituents of Essential oils" (Dr. Detlev Hochmuth, Scientific consulting, Hamburg, Germany) using the MassFinder 3 software (www.massfinder.com).

2.4. Statistical analysis

Results are expressed as mean of triplicate. One-way analysis of variance (ANOVA) followed by Duncan's multiple range test at the significance level of 5% was used to compare means. Principal component analysis (PCA) was applied to establish the relationship between the essential oil compounds and the site of collection. The SPSS 13.0 (Chicago, Illinois, USA) and the statistical R 2.14.1. software (Wirtschaftsuniversität Wien Augasse, Vienna, Austria) were used to perform statistical analysis.

3. Results and discussion

From the foliage of *C. sempervirens*, pale yellowish oils were obtained with yields of 1.36%, 1.1% and 0.72% for sites 1, 2 and the control, respectively (Fig. 2). These results suggest that the essential oil were intensively synthesized in dusted plants which can be viewed as an adaptive mechanism to cope with

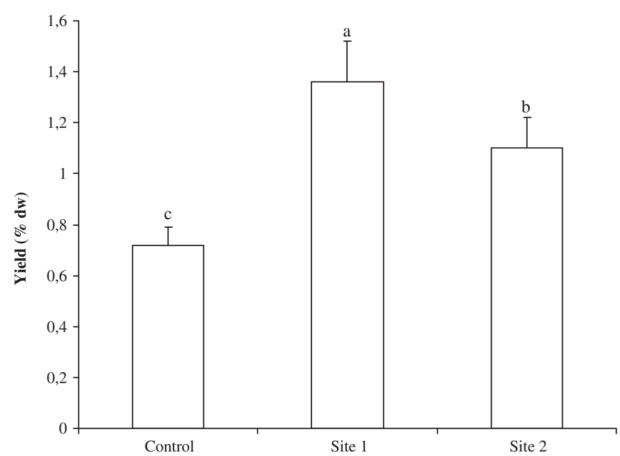

Figure 2 Yields (% dw) of essential oils of *C. sempervirens* leaves collected from unpolluted (control) and polluted sites (site 1 and 2).

the deleterious effects of cement dusts (Dziri and Hosni, 2012) . At this point, it is to be expected that the higher proportion of carbon was allocated to the synthesis and storage of secondary metabolites presumably involved in the chemical defence particularly against cement dust-induced oxidative stress as previously reported in *Schinus areira* (Wannaz et al., 2003), *Lemna minor*, *Ceratophyllum submersum*, *Potamogeton natans* (Erdal and Demirtas, 2010) and *Pinus halepensis* (Dziri and Hosni, 2012).

The identified components and their percentages are given in Table 1, where they are listed in order of their elution from the Rtx-1 column. A total of 54 compounds, covering more than 93% of the total integrated GC peak area were identified. Comparison of the analytical data revealed remarkable qualitative and quantitative differences between plants from

Table 1 Chemical composition (% total peak area) of the leaf essential oils of *C. sempervirens*.

N°	Compounds	RI*	Control	Site 1	Site 2
1	Tricyclene	923	0.2[a]**	0.2[a]	0.2[a]
2	α-Thujene	927	2.2[a]	0.5[b]	0.3[c]
3	α-Pinene	930	48.1[b]	62.2[a]	63.8[a]
4	Camphene	938	2.4[a]	0.9[c]	1.3[b]
5	α-Fenchene	942	–	tr	–
6	Sabinene	958	0.6[b]	0.9[a]	–
7	1-Octen-3-ol	960	–	1.3[b]	1.7[a]
8	β-Pinene	980	2.8[a]	2.1[b]	2[b]
9	*Z*-hex-3-enyl-acetate	990	–	tr	–
10	α-Phellandrene	1001	tr	0.2[a]	–
11	δ-3-Carene	1003	24.4[a]	17.5[b]	23.1[a]
12	α-Terpinene	1006	0.1[a]	0.1[a]	–
13	*p*-cymene	1012	0.1[b]	0.4[a]	0.4[a]
14	Limonene	1015	1.2[b]	2[a]	1.9[a]
15	(*E*)-β-ocimene	1037	0.4[a]	0.2[b]	–
16	γ-Terpinene	1044	0.4[a]	0.4	–
17	Nonan-2-one	1067	–	tr	–
18	*p*-cymenene	1072	2.7[a]	2.7[a]	2.6[a]
19	Borneol	1152	–	tr	–
20	Pulegone	1215	0.2	tr	–
21	*Cis*-chrysanthenyl acetate	1241	–	tr	–
22	Carvacrol	1279	1.8[a]	0.2[b]	0.2[b]
23	Undecan-2-ol	1284	0.9	1.3	1.3[a]
24	(*E*-*E*)-2.4-decadienal	1281	–	tr	–
25	Myrtenyl acetate	1318	–	tr	–
26	α-Cubebene	1347	–	tr	–
27	β-Bourbonene	1384	–	0.1[a]	–
28	β-Elemene	1388	0. 9[a]	0.1[b]	–
29	β-Isocomene	1405	–	tr	–
30	β-Caryophyllene	1422	–	tr	–
31	β-Copaene	1427	0.3	0.1[b]	–
32	Aromadendrene	1436	–	0.1	–
33	α-Humulene	1454	0.5[b]	1.2	0.3[c]
34	γ-Muurolene	1469	–	0.1[a]	–
35	Germacrene-D	1478	–	0.1[a]	–
36	γ-Humulene	1487	–	0.1[a]	–
37	Bicyclogermacrene	1494	–	tr	–
38	(*E.E*)-α-farnesene	1499	1[a]	0.2[b]	–
39	α-Calacorene	1522	–	tr	–
40	*E*-Nerolidol	1543	–	tr	–
41	β-Calacorene	1548	–	tr	–
42	Spathulenol	1558	0.6[b]	2.1[a]	0.6[b]
43	Caryophyllene oxide	1567	–	tr	–
44	Globulol	1575	–	tr	–
45	Tridecanol	1581	–	tr	–
46	Viridiflorol	1591	–	0.1[a]	–
47	Humulene epoxide II	1595	–	tr	–
48	8-*epi*-γ-Eudesmol	1606	–	0.1[a]	–
49	*Epi*-cubenol	1613	–	tr	–
51	γ-Eudesmol	1617	0.2[a]	0.1[a]	–
51	α-Cadinol	1644	–	0.1[a]	–
52	Hexanoic acid	1946	0.5[a]	0.3[b]	0.2[c]
53	Manoyl oxide	2002	tr	0.2[a]	–
54	E-Phytol	2096	1[a]	0.4[b]	–
	Monoterpene hydrocarbons		85.5[c]	90.1[b]	95.5[a]
	Oxygenated monoterpenes		1.9[a]	0.3[b]	0.2[b]
	Sesquiterpene hydrocarbons		2.7[a]	2.1[a]	0.3[b]
	Oxygenated sesquiterpenes		0.8[b]	2.6[a]	0.6[b]
	Others		2.3[b]	3.5[a]	3.2[a]
	Total identified		93.2	98.71	99.92

* RI: retention indices relative to n-alkanes on the apolar column Rtx-1; tr: trace (<0.05%); –: not detected.
** Values within rows followed by the same letter are not significantly different at *p* (5%).

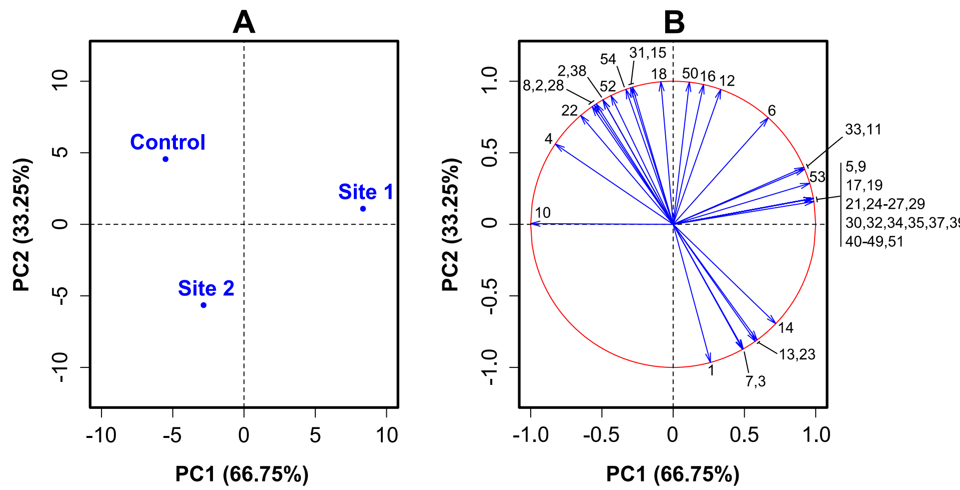

Figure 3 Principal component analysis (PCA) of A: site of collect and, B: the leaf essential oils of *C. sempervirens*.

polluted and unpolluted (control) sites. The most complex composition (54 compounds) was recorded for the oil collected from the heavily polluted site (Site 1), whereas, plant essential oils from site 2 and control site were less complex (15 and 26 compounds for site 2 and control site, respectively). Differences in the number of identified compounds suggest that numerous metabolic pathways were elicited in the secondary metabolism of *C. sempervirens* under atmospheric pollution caused by cement dusts.

Considering the main chemical classes, the most plentiful compounds were monoterpene hydrocarbons (85.5–95.5%) with α-pinene (48.05–63.75%) and δ-3-carene (17.54–24.43%) being the main components. The former compound was particularly abundant in plants from site 1 while the second compound was abundant in plants from the control site. Remarkable increase in the amount of some monoterpene hydrocarbons such as sabinene, *p*-cymene and limonene were observed in the plant growing closest to the cement factory. The reciprocal trend was observed for α-thujene, camphene, β-pinene, (*E*)-β-ocimene, γ-terpinene and *p*-cymenene.

For oxygenated monoterpenes, the highest contribution of this fraction was found in plants from the unpolluted site (1.95%) while the lowest was observed in plants from site 2 (0.24%). The same trend was also observed for sesquiterpene hydrocarbons which were more abundant in plants from the unpolluted site (2.7%). In contrast, oxygenated sesquiterpenes with spathulenol as the most prominent compounds were particularly abundant in the oils from plants collected in the heavily polluted site 1. Other components mainly 1-octen-3-ol and undecan-2-ol showed the same pattern being more represented in the oils from plants of the polluted sites. In order to shed more light on the chemical variability of the essential oils acquired at different sites (Dziri and Hosni, 2012), a PCA was applied to all identified constituents. The scatter plot, obtained by PCA (Fig. 3a, b) showed a great variability in the essential oil chemical composition, with 100% cumulative variance in the two factorial plans. The first principal component (PC 1), accounting for 66.75% of total variance allows distinguishing the oil from the heavily polluted site (site 1) which is characterized by its relatively higher contents of sesquiterpenes (α-humulene, spathulenol, etc.), diterpene manoyl oxide and other compounds namely 1-octen-3-ol which could be considered as indicators of cement dust pollution. The second PC 2, accounting for further 33.25% allowed clear separation of the oil from site 2, characterized by its higher amounts of monoterpene hydrocarbons (tricyclene, α-pinene, p-cymene, δ-3-carene and limonene) and other components such as undecan-2-ol.

In general, our results revealed significant changes in qualitative and quantitative chemical composition of the essential oils of *C. sempervirens* in response to cement dusts. The obvious variations in the percent contribution of group components as well as individual component imply that the biosynthesis and storage of monoterpene hydrocarbons in plants exposed to cement dusts receive relatively high priority (Dziri and Hosni, 2012). Support to this assumption is given by Judzentiene et al. (2007) and Kupcinskiene et al. (2008), who reported that the exposure of Scots pine (*P. sylvestris*) to cement dusts resulted in increased amount of monoterpene hydrocarbons. They also showed that the magnitude of variations in monoterpene content depends on the distance from the cement factory and the age of plant. A redirection of the secondary metabolism of Aleppo pine (*P. halepensis*) towards the biosynthesis of monoterpene hydrocarbons in response to cement dust has been recently reported (Dziri and Hosni, 2012). Such metabolic feature can be explained by the fact that the biosynthesis of monoterpenes requires relatively low molecular resources (precursors) and enzymatic activity, which make it energetically feasible compared to that of sesquiterpenes which requires more biological precursors, enzymatic reactions namely oxidations, reductions, condensations, isomerization, cyclizations, etc. (Baby et al., 2010).

In addition to this metabolic scheme, the dynamic accumulation of monoterpenes in dusted plants might be a direct consequence of the inhibition of emission of biogenic volatile compound following the stomatal closure, leading hence to an increased amount of stored monoterpene in the storage cavities of *C. sempervirens*, considered as a monoterpene-storing species and a stronger emitter of biogenic volatile compounds (Loreto and Schnitzler, 2010).

From a physiological perspective, this chemical changes in the metabolism of terpenes may be interpreted as an attempt to

cope with the deleterious effects induced by cement dusts. Confirmation of this assumption is reflected in the opposite changes in the amount of the main components α-pinene and δ-3-carene which has been considered as defensive chemicals against oxidative stress induced by air pollution (Shpak et al., 2007). These authors also reported increased amount of β-pinene, δ-3-carene and terpinolene in *P. sylvestris* growing in radionuclide contaminated areas, which is in good agreement with our results. The same species when exposed to cement dusts exhibited higher amount of δ-3-carene, γ-terpinene, terpinolene and α-terpinene (Kupcinskiene et al., 2008). The protective mechanism mediated by monoterpene has also been reported in *S. areira* exposed to gaseous SO_2 (Wannaz et al., 2003). It has been proposed that monoterpenes were involved in stabilizing cell membranes *via* lipid–lipid, lipid–protein or protein–protein interactions and/or by scavenging the reactive oxygen species (ROS) conferring hence, better protection from environmental constraints (i.e. ozone, atmospheric CO_2, higher temperature, drought, salinity, UV radiations, and air pollution among others) (Hartikainen et al., 2009; Holopainen, 2011).

However, because of the extremely complicated environmental situations in the area surrounding the cement factory (high CO_2 concentration, high concentration of SO_x and NO_x, alkaline soils etc.), it is not always clear which pollutant induces changes in the chemical composition of the essential oil. Nevertheless, literature data could provide key elements to explain at least partly the results obtained herein. For example, Räisänen et al. (2008) demonstrated that the long term exposure to elevated CO_2 concentrations and higher temperatures resulted in remarkable increase of α-pinene and decrease of δ-3-carene in *P. sylvestris*. Similar results were also found one year earlier in the same species growing in radionuclides contaminated areas (Shpak et al., 2007). They attributed this trend to the increased reactivity of δ-3-carene in oxidative processes, which increase with increasing contamination. Growing under elevated ozone (O_3), plant of European aspen (*Populus tremula*) showed increased amount of α-pinene (Hartikainen et al., 2009). Three years later, the same authors have evaluated the impact of elevated temperature and ozone on the emission of volatile organic compounds (VOCs) in silver birch (*Betula pendula*) and have pinpointed that the increased emission of VOCs in response to elevated ozone was the direct consequence of the enhancement of the transcription of genes encoding terpenes synthesis namely 1-deoxy-D-xylulose 5-phosphate synthase (DXS), 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR) and isopentenyl diphosphate (*IPP*) isomerase (Hartikainen et al., 2012). In contrast, the emission of monoterpenes was completely repressed when the ozone concentrations surpass the threshold required (150–300 ppm) (Loreto and Schnitzler, 2010).

Taken together, it appears that the response of a plant to contamination depends on the plant species, nature of pollutant and the residence time of the pollutant.

4. Conclusions

This is the first study concerning the impact of cement dust on the yield and chemical composition of the essential oil of *C. sempervirens*. Our results clearly indicate that cement dust induced a remarkable increase in the yields of the essential oils and induces qualitative and quantitative changes in their chemical composition. The cement dust related increase in the yield is potentially associated with increase of the monoterpene, particularly α-pinene content. In addition, from an ecological point of view, *C. sempervirens* could represent an excellent alternative for the creation of green areas around cement factory due to its relative tolerance to air pollution caused by cement dust. From a commercial standpoint, these results may be significant given the economic importance of monoterpenes for the fragrance, flavour and pharmaceutical industries.

Acknowledgments

The authors are indebted to the Direction Générale de la Recherche Scientifique (DGRST, Tunisia) and the Centre National de la Recherche Scientifique (CNRS, France) and the Laboratoire International Associé (LIA) for financial support (Research Project PHC-Utique N° 13G0904).

References

Abrahim, D., Francisckini, A.C., Pergo, E.M., Kelmer-Bracht, A.M., Ishii-Iwamoto, E.L., 2003. Effects of α-pinene on the mitochondrial respiration of maize seedlings. Plant Physiol. Biochem. 41, 985–991.

Adams, R., 2001. Identification of essential oil components by gas chromatography/quadrupole mass spectroscopy. Allured, Carol Stream, IL, USA.

Aharoni, A., Jongsma, M.A., Bouwmeester, H.J., 2005. Volatile science? Metabolic engineering of terpenoids in plants. Trends Plant Sci. 10, 594–602.

Baby, S., Raj, G., Thaha, A.R.M., Dan, M., 2010. Volatile chemistry of a plant: monosesquiterpenoid pattern in the growth cycle of *Curcuma haritha*. Flavour Fragr. J. 5, 35–40.

Bačić, T., Lynch, A.H., Cutler, D., 1999. Reactions to cement factory dust contamination by *Pinus halepensis* needles. Environ. Exp. Bot. 41, 155–166.

Bae, G.-S., Park, K.-C., Choi, S.B., Jo, I.-J., Choi, M.-O., Hong, S.-H., Song, K., Song, H.-J., Park, S.-J., 2012. Protective effects of alpha-pinene in mice with cerulean-induced acute pancreatitis. Life Sci. 91, 866–871.

Bakkali, F., Averback, S., Averback, D., Idaomar, M., 2008. Biological effects of essential oils – a review. Food Chem. Toxicol. 46, 446–475.

Banchio, E., Zygaldo, J., Valladares, G.R., 2005. Quantitative variations in the essential oil of *Minthostachys mollis* (Kunth.) Griseb. In response to insects with different feeding habits. J. Agric. Food Chem. 53, 6903–6906.

Chang, H.-T., Cheng, Y.-H., Wu, C.-L., Chang, S.-T., Chang, T.-T., Su, Y.-C., 2008. Antifungal activity of essential oil an dits constituents from *Calocedrus macrolepis* var. *formosana* Florin leaf against plant pathogenic fungi. Bioresour. Technol. 99, 6266–6270.

Cool, L.G., Hu, Z.-L., Zavarin, E., 1998. Foliage terpenoids of Chinese *Cupressus* species. Biochem. Syst. Ecol. 26, 899–913.

De Alwis, R., Fujita, K., Ashitani, T., Kuroda, K., 2009. Volatile and non-volatile monoterpenes produced by elicitors-stimulated *Cupressus lusitanica* cultured cells. J. Plant Physiol. 166, 720–728.

Dziri, S., Hosni, K., 2012. Effects of cement dust on volatile oil constituents and antioxidative metabolism of Aleppo pine (*Pinus halepensis*) needles. Acta Physiol. Plant. 34, 1669–1678.

Erdal, S., Demirtas, A., 2010. Effects of cement flue dust from a cement factory on stress parameters and diversity of aquatic plants. Toxicol. Ind. Health 26, 339–346.

Harrathi, J., Hosni, K., Karray-Bouraoui, N., Attia, H., Marzouk, B., Magné, C., Lachaâl, M., 2012. Effect of salt stress on growth, fatty acids and essential oils in safflower (*Carthamus tinctorius* L.). Acta Physiol. Plant. 34, 129–137.

Hartikainen, K., Nerg, A.-M., Kivimäenpää, M., Kontunen-Soppela, S., Mäenpää, M., Oksanen, E., Rousi, M., Holopainen, T., 2009. Emission of volatile organic compounds and leaf structural characteristics of European aspen (*Populus tremula*) grown under elevated ozone and temperature. Tree Physiol. 29, 1163–1173.

Hartikainen, K., Riikonen, J., Nerg, A.-M., Kivimäenpää, M., Ahonen, V., Tervahauta, A., Kärenlampi, S., Mäenpää, M., Rousi, M., Kontunen-Soppela, S., Oksanen, E., Holopainen, T., 2012. Impact of elevated temperature and ozone on the emission of volatile organic compounds and gas exchange of silver birch (*Betula pendula* Roth). Environ. Exp. Bot. 84, 33–43.

Holopainen, J.K., 2011. Can forest trees compensate for stress-generated growth losses by induced production of volatile compounds? Tree Physiol. 31, 1356–1377.

Hosni, K., Zahed, N., Chrif, R., Abid, I., Medfei, W., Kallel, M., Ben Brahim, N., Sebei, H., 2010. Composition of peel essential oils from four selected Tunisian *Citrus* species: evidence for the genotypic influence. Food Chem. 123, 1098–1104.

Hosni, K., Hassen, I., M'Rabet, Y., Sebei, H., Casabianca, H., 2013. Genetic relationships between some Tunisian Citrus species based on their leaf volatile oil constituents. Biochem. Syst. Ecol. 50, 65–71.

Judzentiene, A., Stikliene, A., Kupcinskiene, E., 2007. Changes in essential oil composition in the needles of Scots pine (*Pinus sylvestris* L.) under anthropogenic stress. Sci. World J. 7 (S1), 141–150.

Kupcinskiene, E., Stikliene, A., Judzentiene, A., 2008. The essential oil qualitative and quantitative composition in the needles of *Pinus sylvestris* L. growing along industrial transects. Environ. Pollut. 155, 481–491.

Jiang, Y., Wu, N., Fu, Y.J., Wang, W., Luo, M., Zhao, C.-J., Zu, Y.-G., Liu, X.-L., 2011. Chemical composition and antimicrobial activity of the essential oil of rosemary. Environ. Toxicol. Pharmacol. 32, 63–68.

Lima, F.J.B., Brito, T.S., Freire, W.B.S., Costa, R.C., Linhares, M.I., Sousa, F.C.F., Lahlo, S., Leal-Cardoso, J.H., Santos, A.A., Magalhães, P.J.C., 2010. The essential oil of *Eucalyptus tereticornis*, and its constituents α- and β-pinene, potentiate acetylcholine-induced contractions in isolated rat trachea. Fitoterapia 81, 649–655.

Loreto, F., Schnitzler, J.-P., 2010. Abiotic stresses and induced BVOCs. Trends Plant Sci. 15, 154–166.

Mahmoud, S.S., Croteau, R., 2002. Strategies for transgenic manipulation of monoterpenes biosynthesis in plants. Trends Plant Sci. 7, 366–373.

Matsuo, A.L., Figueiredo, C.R., Arruda, D.C., Pereira, F.V., Scutti, J.A.B., Massaoka, M.H., Travassos, L.R., Sartorelli, P., Lago, J.H.G., 2011. α-Pinene isolated from *Schinus terebinthifolius* Raddi (Anacardiaceae) induces apoptosis and confers antimetastatic protection in a melanoma model. Biochem. Biophys. Res. Commun. 411, 449–454.

Ojeda-Sana, A.M., van Baren, C.M., Elechosa, M.A., Juárez, M.A., Moreno, S., 2013. New insights into antibacterial and antioxidant activities of rosemary essential oils and their main components. Food Control 31, 189–195.

Paolini, J., Costa, J., Bernardini, A.-F., 2005. Analysis of the essential oil from aerial parts of *Eupatorium cannabinum* subsp. *corsicum* (L.) by gas chromatography with electron impact and chemical ionization mass spectrometry. J. Chromatogr. A 1076, 170–178.

Phillips, M.A., León, P., Boronat, A., Rodríguez-Concepción, M., 2008. The plastidial MEP pathway: unified nomenclature and resources. Trends Plant Sci. 13, 619–623.

Räisänen, T., Ryyppö, A., Julkumen-Tiitto, R., Kellomäki, S., 2008. Effects of elevated CO_2 and temperature on secondary compounds in the needles of Scots pine (*Pinus sylvestris* L.). Trees 22, 121–135.

Shpak, S.I., Lamotkin, S.A., Lamotkin, A.I., 2007. Chemical composition of *Pinus sylvestris* essential oil from contaminated areas. Chem. Nat. Compd. 43, 55–58.

Wang, W., Wu, N., Zu, Y.G., FU, Y.J., 2008. Antioxidative activity of *Rosmarinus officinalis* L. essential oil compared to its main components. Food Chem. 108, 1019–1022.

Wannaz, E.D., Zygaldo, J.A., Pignata, M.L., 2003. Air pollutants effects on monoterpenes composition and foliar chemical parameters in *Schinus areira* L. Sci. Total Environ. 305, 177–193.

Yang, N.-Y., Zhou, G.S., Tang, Y.-P., Yan, H., Guo, S., Liu, P., Duan, J.-A., Song, B.-S., He, Z.-Q., 2011. Two new α-pinene derivatives from *Angelica sinensis* and their anticoagulative activities. Fitoterapia 82, 692–695.

Yilmaz, S., Zengin, M., 2004. Monitoring environmental pollution in Erzurum by chemical analysis of Scot pine (*Pinus sylvestris* L.) needles. Environ. Int. 29, 1041–1047.